UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MAR 1, 2007

MEMORANDUM

SUBJECT: Science review of EMD-004.3 report of completed efficacy study of IR3535 Aerosol Spray formulation against mosquitoes.

FROM: Clara Fuentes, Ph.D., Biologist
Biochemical Pesticides Branch
Biopesticides & Pollution Prevention Division (7511P)

TO: Linda Hollis, Branch Chief
Biochemical Pesticides Branch
Biopesticides & Pollution Prevention Division (7511P)

REF: Carroll, S. (2006) Test of Personal Insect Repellents (Aerosol Spray). Unpublished study conducted by Carroll-Loye Biological Research under Project No. EMD-004.3. 147 p. (MRID 47045902)

ACTION REQUESTED

Provide scientific review of the completed study, MRID 47045902 EMD-004.3 Aerosol Spray formulation, to evaluate its scientific validity and assess its consistency with changes recommended by EPA and HSRB to the revised protocols

CONCLUSIONS

I have reviewed Carroll-Loye product performance study, MRID 47045902 EMD-004.3 Aerosol Spray formulation, containing 20% w/w of the active ingredient IR3535, and concluded that the study, EMD-004.3, provides information sufficient for evaluating the repellent properties of this formulation against mosquitoes. The reported complete protection time (CPT) ranges from 8.75 to 9.75 hours (Mean CPT = 9.65 ± 0.32 hours) for the wooded picnic area. The CPT for the Native grassland site, was 10.25 hours; no subjects experienced LIBes during the test period. (Table 6, p. 18).

The reported study, MRID 47045902 EMD-004.3 Aerosol Spray, is scientifically sound and able to generate reliable data for evaluating the repellency of the formulation tested against mosquitoes. This study was conducted consistently with changes made to the revised protocol as recommended by EPA and HSRB. These changes are listed below:

1. Addition of preliminary phase to estimate typical consumer dose
2. Discussion on risk and risk minimization
3. Discussion of sample size and statistical analysis
4. Elimination of positive controls
5. Pre-test training for dosimetry and product performance testing
6. Change repellency endpoint to FCLIBe
7. Reduce number of negative controls to 2 subjects for assessment of biting pressure
8. Monitor test sites for incidence of West Nile virus (WNV) prior to conducting the test

Next are the HSRB specific recommendations to the amended study protocol:

1. Conduct the dosimetry test outdoors for the spray formulation.
2. Determine effective dose
3. Ensure safety of test material including information on toxicological reference points such as NOAEL/LOAEL

The following are the protocol changes and recommendations adopted in the performance of EMD-004.3 study:

1. Dosimetry test was conducted outdoors. "Applications were made out of doors, immediately adjacent to the laboratory." (p. 9).
2. Information concerning acute low toxicity of the test material is available.

   P.40 *Study Protocol*. 6.1.7 *Test Material Safety*
   "The insect repellent products proposed for registration have all been tested in animals for potential oral and dermal toxicity, dermal inhalation, ocular and dermal sensitization potential; studies on droplet size of spray and aerosol products showed that there was little if any potential for inhalation exposure. These studies will be submitted and reviewed by EPA as part of the registration process. The results of these tests showed a low order of toxicity characteristic of similar tests on the "neat" active ingredient cited by EPA in approvals of this product for application on humans. The IR3535 active

ingredient has an extensive, positive safety record in consumer use. MSDS documentation is the same as that submitted with the previous version of this protocol."

3. The study provides justification for sample size, and discussion of statistical procedures for analysis of dosimetry and repellency data.

   "Twelve human subjects were used in measurements of self-dosing behavior. Ten human subjects exposed the test material to mosquitoes for efficacy evaluation. A sample size of ten subjects was chosen for efficacy testing to give a reasonably large statistical population size while avoiding exposing too many individuals to the minor but present risks associated with exposure to biting arthropods." p. 7.

4. Risk from exposure to formulation was further minimized by reducing the number of unnecessary exposures from 3 to 1 during preliminary practice prior to initiation of dosimetry test.

   "After practicing applying the Aerosol once to each limb to get a feel for its dispensing properties, subjects completed a series of three self application replicates to each limb." p. 9.

5. Risk from exposure to mosquito's bites and mosquito borne diseases were adequately minimized as summarized below:

   a) The efficacy endpoint was changed to first confirmed landing with intent to bite (FCLIBe)

      P. 36: 5.1 *Objective of Research Protocol EMD-004*

      "Complete Protection Time, or CPT, is defined herein as the time between application of test material and First Confirmed Lite with Intend to Bite. A lite with intend to bite, or LIBe, occurs when a mosquito alights on the treated test skin of a subject and extends its proboscis to the skin surface while ceasing locomotion. A First Confirmed LIBe is that which is followed by another within 30 minutes." P. 38: 5.4 *Balance of Risks and Benefits. Protocol EMD-004*.

   b) Exposure periods were limited to 1 minute every 15 minutes.

      "Data were recorded by the Study Director every 15 minutes, after each one-minute exposure." p. 11.

      "Exposures took place at 15 minutes intervals, which began 15 minutes after applications of the test material at the Forest site, and approximately 3 hours and 15 minutes after applications at the Marsh/Pasture site. A technician advised subjects when the 1 minute [exposure] period began and ended." p. 11.

Reviewer comment:

The last paragraph on p. 11 states that data from first exposures were recorded as taking place at 2 hours after application of test material at site 2 (Forest site). This is inconsistent with the statement in the above paragraph, where it says that the exposures began 15 minutes after applications [of the test material] at the Forest site.

Dr. Carroll has responded to this comment via e-mail dated 3-1-07 addressing the following: ". . . [I]n section 7 (p. 11) the report states that applications were made 3:15 in advance at the marsh site, but only 15 minutes in advance at the forest site. However, as shown in Appendix 2, applications for the Forest site were made 2 hours in advance of the first exposure. I will submit a formal correction for that typo." (copy of the e-mail is attached).

c) Prior to testing, field sites were monitored for detection of mosquito-borne pathogens.

"Field tests are being conducted in an area where such viruses [West Nile Virus] have not been detected by County and state health or vector/mosquito control agencies for at least a month" Page 38 *Protocol EMD-004*.

"Sites were chosen based on surveillance data compiled by the Californian State Department of Health Services. . . . Mosquitoes were engaged as encountered in nature. Sites were chosen based on surveillance data compiled by the California State Department of Health Services. Our goal was to find sites with active nuisance mosquito populations from which West Nile (WNV) or related virus had not been recently isolated. Counties in the Central Valley of California generally sustain large populations of mosquitoes late in the year, making the Valley one of the only areas in the United States suitable for mosquito efficacy testing as winter approaches. However, incidence of West Nile and other encephalopathic viruses generally declines to zero in autumn. No sentinel chicken flocks recorded WNV after September in Merced Co. One sentinel chicken flock had a single positive for WNV in the Butte County region in the month preceding our work, but flocks closer to our sites had not. Importantly, a mid-October survey of several thousand mosquitoes in areas close to our Butte Co. site revealed no presence of WNV in tests by the US Centers for Disease Control (personal communication from Ms. Debra Lemanager, Vector Ecologist, Sutter Yuba Mosquito Abatement District, Yuba City, California). Ms. Lemanager regarded WNV activity in Northern
California as being effectively concluded for 2006." Page 8.

d) Test subjects were pre-trained in the laboratory to handle mosquitoes using mechanical aspirators. The mosquitoes used for pre-training were laboratory reared pathogen-free mosquitoes.

"[S]ubjects will have approximately one hour of training and practicing observing foraging mosquitoes and catching them from their own arms in a laboratory cage, using an aspirator . . . . The mosquitoes used for training are *Aedes aegypti* reared in the laboratory and free from diseases." Page 60 *Protocol EMD-004*.

e) In the field, test subjects were arrayed in pairs to facilitate removal of mosquitoes "with intent to bite" and data collection.

"Treated subjects were partnered into groups of two. Each member of a partner pair was instructed to monitor their own exposed limb and that of their partner for mosquito landings during one-minute periods of exposure to mosquitoes (a "buddy system")" p. 11

f) Reduction in number of negative control subjects to 2 experienced personnel, attended by 2 assistants.

"Ambient LIBe pressure was measured by 2 experienced personnel on the same schedule as that for repellent exposure. These negative control subjects were attended by 2 assistants who use mechanical aspirator …" page 11.

6. Deviations from the protocol

"5. Sponsor concerns about the formulation of the aerosol led us to remove that product from the efficacy trials" page 30.

Reviewer comment:

The above statement referring to *Deviations from the Protocol* does not correspond to this study report, which is for evaluating the performance of the Aerosol Spray formulation. Dr. Carroll responded to this comment via e-mail message, dated 2-28-07:

"First, the deviation statement regarding excluding the aerosol is should not be in this report. It is left over from the previous reports. Clearly this is the aerosol study report.

Second, the other deviations listed apply to this report.

Third, an additional "planned deviation" should be listed: because we were conducting the testing later in the season that originally anticipated, we needed to find a way to deal with how the short autumnal day length reduced our daily window of exposure time to mosquitoes. Given that one field study sites were a 2-3 hour drive from our laboratory, instead of applying the aerosol on arrival at the study sites, we applied the test material at the lab, before traveling to the study sites. Doing so allowed us to test farther into the period of likely failure. The prior studies with lotion and pump spray had given us reason to anticipate no failure in the first few hours after application, with a significant increase in failure

probability several hours after application. In fact, those test results, which showed a remarkably long duration of protection, highlighted the importance of ensuring sufficient exposure time during daylight hours (when observations can be readily made). Accordingly, this design modification to accommodate seasonal changes in day length made the test more comparable to typical summer tests in which the day photoperiod is longer."

STUDY SUMMARY:

MRID 47045902

A Establishment of typical consumer dose

The weight of material applied during dosimetry was measured using 2.5 cm wide strips of self adhesive roll gauze bracelets, evenly spaced across the leg. Bracelets were weighed before and after each application, and the formulation container was also weighed before and after each application. Each subject repeated the application procedure 3 times. The 12 individual means of 3 applications per subject were averaged across 12 subjects, and "the grand mean of subjects means was then used as the dosage rate for the efficacy testing. Those applications were made volumetrically, based on the limb surface areas of each subject and the specific gravity of the Aerosol repellent (0.94 g/ml; Appendix 9)." page 9.

"[T]o determine dosage, we measured lower limbs surface area for individual subjects based on the length and a set of 4 circumferences taken from each limb…A mean dosage weight was calculated for each subject based on the weight increment of the dosimeters, multiplied by the quotient of the limb surface area divided by the dosimeter surface area. This analysis yield a dosing rate of g/ cm^2, and was calculated for 7 females and 5 male subjects." (p. 9)

"…the average weight increment in treated dosimeters was 0.29 ± 0.14 g (p. 13).

"The mean (± sd) dosing rate for legs was 0.000987 (approximately 0.0010) ± 0.000055 g/cm^2. Based on that mean and the specific gravity of the Aerosol repellent (0.94, Appendix 9), we used a dosing rate of 0.00105 ml/cm^2 for the subsequent efficacy test." (p. 15).

The average dosing rate used for efficacy testing was 0.001 ml/cm^2. Individual doses were prepared for each subject based on the surface area of their lower legs (p. 10) (Refer to Appendix 3. *Treatment Allocation and Dosing*, p. 26).

Calculations:

The estimated dosage per trial = **Total captured** x 1/ **Proportion covered**,
where:

Total captured = difference in mass (gain or loss of weight) between treated and untreated dosimeters.

Proportion of total surface area covered by dosimeter = surface area of a set of 4 dosimeters / surface area of the limb

Dosing rate (in weight) / surface area of skin = total estimated weight of applied material per limb surface area. These values were converted to volume by dividing the weight in grams by the specific gravity of the formulation :

Mean dosing rate [g/ cm^2] divided by test material specific gravity [g/ml] = Efficacy test dosing rate [ml/ cm^2]

B. Product performance

Study sites were 2 different habitats in the state of California, where the mosquito population was monitored for the presence of pathogen vectors prior to conducting the study, and found negative to WNV. The efficacy endpoint was changed to FCLIBe. Sample size of 10 replications (subjects) per treatment was justified and used to estimate the average CPT.

Risk minimization included pre-test training on handling pathogen-free mosquitoes in the laboratory; intermittent exposures of 1 minute to mosquitoes in the field every 15 minutes; arrangement of test subjects in pairs to assist each other with data collection, and reduction of negative control subjects to 2 experienced personnel, attended by 2 assistants. Test sites were monitored prior to testing for lack of pathogen vectors in those localities. Results generated from these efficacy studies were analyzed as proposed in the revised protocol. Data was analyzed using descriptive statistics (SAS JMP version 5.0.1.2 SAS Institute, Cary NC). Mean CPT was calculated with its associated standard deviation. The reported complete protection time (CPT) for the Aerosol Spray formulation in the forest site was CPT= 9.75 ± 0.32; for the marsh/pasture site, CPT was 10.25 hours for all subjects (Table 6. p. 18).. .

REVIEWER COMMENTS

Dosimetry is one of the strengths of the revised protocol, which is used to verify subjects' safety. The reported study tested the Aerosol Spray formulation outdoors for determination of dosage as recommended by the HSRB.

Risk minimization of subjects' exposure to the test material and tick bites during testing were adequately addressed for both dosimetry and product performance tests. Risk minimization approaches include a deviation from the revised protocol by reducing the number of applications of formulation per subject from 3 to 1 during pre-test practice, and pre-test training using aspirators to capture laboratory reared, pathogen-free mosquitoes in the laboratory as proposed in the revised protocol. The study report also addresses availability of data on acute toxicity and safety of the test material submitted with the original protocol. The test material has been tested

for acute toxicity on animals. These data show low toxicity. MSDS documentation for the active ingredient is included in the original study protocol. Thus, the studies adopted HRSB recommendations concerning information on toxicological reference points such as NOAEL/LOAEL for safety of test material during dosimetry trials.

Repellency endpoint was changed to FCLIBe. Minimization of risk from exposure to mosquito bites and pathogen transmission were adequately addressed in the study method.

Sample sizes were 12 and 10 subjects for dosimetry and product performance tests, respectively, as discussed in the revised protocol.

Data analysis "Dosimetry analysis was based on subjects means, consisted of non-parametric rank and correlation test, and parametric regression. Those, and other, descriptive statistics were generated with the software 'SA JMP' Version 5.0.12 (SAS Institute, Cary NC)"… "Mean CPT was calculated across all 10 subjects, and is presented with standard deviation and 95% confidence interval information as well. (p. 12)

Protocol deviations while conducting the studies are adequately documented.

Attachments:

Dr. Carroll's emails dated 3-1-07 and 2-28-07

From: **Scott P Carroll <spcarroll@ucdavis.edu>** 03/01/2007 12:38 PM
To: Clara Fuentes/DC/USEPA/US@EPA
Subject: Re: EMD-004.3 and EMD-003.3

Hi Clara,

As you know I was travelling yesterday, and so I am sorry I could not immediately reply to your last message from yesterday.

I have now had time to review our documentation in greater detail in order to respond to your questions.

As I initially stated in my telephone message yesterday, we made the applications to the subjects in advance of travel to both field sites in Study 004.3. In reviewing the report briefly thereafter, I noted that in section 7 (p 11) the report states that applications were made 3:15 in advance at the marsh site, but only 15 minutes in advance at the forest site. However, as shown in Appendix 1 and Appendix 2, applications for the Forest site were made 2 hours in advance of the first exposure.

I will submit a formal correction for that typo and/or proceed as you advise.

Thanks very much. Perhaps we should talk by telephone if that would make it easier to discuss the calculations to which you refer.

Best,
Scott

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Scott P. Carroll, PhD
Department of Entomology
Center for Population Biology
University of California-Davis
Davis, CA 95616

Tel (530) 297-6080
http://www.carroll-loye.com/

From: **Scott P Carroll <spcarroll@ucdavis.edu>** 02/28/2007 04:21 PM
To: Clara Fuentes/DC/USEPA/US@EPA, Clara Fuentes <fuentesclara@yahoo.com>
Subject: Re: EMD-004.3 and EMD-003.3

Hi Clara,

Thank you for your review. Your comments help to improve the communication of the test results. Thank you for the opportunity to provide you with clarifications.

1. Mosquito/aerosol study EMD-004.3

First, the deviation statement regarding excluding the aerosol is should not be in this report. It is left over from the previous reports. Clearly this is the aerosol study report.

Second, the other deviations listed apply to this report.

Third, an additional "planned deviation" should be listed: because we were conducting the testing later in the season that originally anticipated, we needed to find a way to deal with how the short autumnal day length reduced our daily window of exposure time to mosquitoes. Given that one field study sites were a 2-3 hour drive from our laboratory, instead of applying the aerosol on arrival at the study sites, we applied the test material at the lab, before travelling to the study sites. Doing so allowed us to test farther into the period of likely failure. The prior studies with lotion and pump spray had given us reason to anticipate no failure in the first few hours after application, with a significant increase in failure probability several hours after application. In fact, those test results, which showed a remarkably long duration of protection, highlighted the importance of ensuring sufficient exposure time during daylight hours (when observations can be readily made). Accordingly, this design modification to accommodate seasonal changes in day length made the test more comparable to typical summer tests in which the diel photoperiod is longer.

The time in advance of exposure that applications were made is given on page 11 (section 7). It was 3 hrs 15 min for the marsh pasture site, and 15 minutes (standard) for the forest.

Fourth, I respond to your query regarding dosage calculation. In the protocol, we describe how the total amount applied in calculated for each trial application in the dosimetry part of the study.

$$\text{Total estimated weight of applied material} = \frac{\text{Weight gain of treated dosimeters - weight change in untreated dosimeters}}{\text{surface area of dosimeters/surface area of limb}} \quad (1)$$

Weight change of the untreated (control) dosimeters was negligible, so we treated that as zero.

That left us with

$$\text{Weight gain of treated dosimeters/proportion of arm covered by dosimeters} \quad (2)$$

(The right hand side of equation (1) is just a restatement of the denominator of equation (2))

Implied in the protocol, and more explicitly stated in the report,

$$\text{Dosing rate (in weight) per unit skin surface area} = \text{Total estimated weight of applied material / limb surface area} \quad (3)$$

Each dosimetry subject repeated the application three times, and so we summed each subject's three dosing rates per unit area and divided by 3 to get their subject mean dosing rates.

To get the dosing rate per unit area to be used for efficacy testing, we took the mean of those subject means (i.e. computed the grand mean). All efficacy subjects were then dosed at that grand mean rate per unit area, based on their individual limb surface area.

Those applications for the efficacy trials were made volumetrically, as specified by EPA. In order to convert the dosing rate based on weight, as calculated above, we divided the weight (in grams) by the specific gravity of the test material as provided in the certificate of analysis. Specific gravity is the weight (in grams) of one ml of material. Water is the standard, in that 1 ml of water weighs one gram. The aerosol has a specific gravity slightly less than that of water, so that in order to deliver, e.g., a gram of test material would require the application of slightly more than one ml.

The final equation then, is:

Efficacy test dosing rate (ml/square cm) =
Mean of subject mean dosing rate (g/square cm) / test material specific gravity (g/ml) (4)

Fifth, your question about liting pressure (similarity of mosquito species across habitats). There are interrelated points to be made here. Most basically, we work with the mosquitoes available, aiming to get appropriately high biting pressure and a representation of species relevant to public health and nuisance concerns. The suite of mosquito species we tested against is more diverse that typically reported for field efficacy studies. *Aedes melanimon* is abundant in California in autumn and tends to pervade a number of habitats in that season. Other genera and species were present, in particularly high numbers late in each test day. Those other species provided most of the liting pressure during the last hour of the tests, when the repellent was potentially most compromised in performance due to the time elapsed since application. More than one mosquito approached the untreated arms limbs during most exposure period, but those were not quantified beyond the single mosquito observed that signaled the cessation of exposure. Particularly in the late exposures, each subject was surrounded by perhaps dozens of mosquitoes, of several species. Yet in general they were fully protected.

2. Tick aerosol study EMD-003.3

Regarding exposure duration, subjects exposed for from 4.25 to 14 hours. Because this was a lab test, we were not dependent on field day length, and so we did not have to carry out advance applications in the same manner as in 004.3, above.

Please contact me with any additional questions. I appreciate your efforts to have clear communication about these complex reports and am glad to improve that communication further.

Regards,
Scott
(cell) 530-902-8267

Scott P. Carroll, Ph.D.
Carroll-Loye Biological Research
711 Oak Avenue
Davis, CA 95616

Tel (530) 297-6080
Fax (530) 297-6080
email spcarroll@ucdavis.edu

http://www.carroll-loye.com/